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ORIGINAL ARTICLE

Significant association between *MDM2 T309G* polymorphism and colorectal cancer

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Summary

Purpose: *The Murine double minute 2 (MDM2) gene plays a crucial role in regulating and suppressing the function of apoptotic pathway. We investigated the relationship between MDM2 gene SNP309 (T309G) (rs2279744) polymorphism and colorectal cancer (CRC) in a Turkish population.*

Methods: *The polymorphism T309G (rs2279744) in the MDM2 gene was studied in patients with colorectal cancer (n=135) and healthy control subjects (n=145) using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The findings were evaluated using logistic regression and x^2 tests.*

Results: *When CRC cases and controls were evaluated based on different habits and family cancer histories, a statistically significant relationship was found between CRC and alcohol consumption (x^2=4.07, p=0.044). Cancer cases and controls had statistically significant different family histories of cancer (x^2=6.82, p=0.009). There was also significant difference in TG genotype distribution in the MDM2 T309G polymorphism between those with and without cancer (OR=1.98, 95% CI=1.98–3.91, x^2=4.00, p=0.045).*

Conclusions: *The SNP309 polymorphism of the MDM2 gene is associated with increased CRC risk in the Turkish population.*

Key words: *colorectal cancer, polymorphism T309G (rs2279744), MDM2 gene, Turkish population*

Introduction

Colorectal cancer (CRC) is the third most common cancer in the world and the fourth most common cause of death from cancer. An estimated 1,688,780 new cases and 600,920 cancer deaths were reported in 2017 [1]. The mechanisms underlying the development of CRC are complex. Both environmental and genetic factors play important roles in the occurrence and progression of CRC. Genetic epidemiology and twin studies demonstrate that up to 35% of CRC cases may be due to inherited factors, which indicates the importance of inherited genetic susceptibility in carcinogenesis [2]. As a crucial tumor suppressor gene, *p53* plays a critical role in cell cycle regulation, DNA repair, cellular differentiation and apoptosis in many cancers. Variation in this gene may have significant effects on tumor development and cancer risk [3-5].

The *p53* gene is often mutated in malignancies, which highlights its importance in tumor development and progression. *The Murine double minute 2 (MDM2)* gene, on the other hand, is an essential negative regulator of *p53*. When present in excessive amounts, it reduces the activity of *p53* through enhanced proteasomal degradation via ubiquitination pathways [6-8]. It acts with *p53* in

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a feedback loop where *p53* activates *MDM2* at the transcriptional levels while *MDM2* binds, inhibits, and degrades *p53* protein through E3 ligase activity [9-11]. The human *MDM2* gene is located on chromosome 12q13–14, has a genomic size of 34 kb, and contains two promoters, a constituent and a *p53*-responsive intronic promoter [12]. A common polymorphism in the promoter of MDM2, SNP309 (T to G change at nucleotide 309, rs2279744), has been shown to increase the expression of *MDM2* mRNA and protein by altering Sp1-binding affinity, which results in the inhibition of *p53*. Hence, individuals with the *GG* genotype have elevated *MDM2* levels and compromised *p53* function [13]. Additionally, there is a disposition toward earlier cancer inception in patients with the *GG* or *TG* genotype for *MDM2* SNP309 [14]. The *MDM2* rs2279744 polymorphism has been associated with colon, gastric and hepatocellular cancer [15-17].

In a previous study, we investigated the relationship between the *MDM2* gene *T309G* polymorphism on lung, gastric and breast cancer in a Turkish population [18-20]. In the present study, we investigate the association between genetic polymorphisms of the *MDM2* gene and CRC risk in a Turkish population.

Methods

Subjects

This study was approved by the local ethics committee of Cumhuriyet University in Sivas, Turkey, and all patients gave signed informed consent. All subjects agreed to participate and filled in a short questionnaire on their occupation, tobacco use, alcohol consumption, and family history of cancer. In the present study, a total of 135 CRC cases and 145 healthy controls were included and studied. Blood samples were collected from the 135 cases that were diagnosed as having CRC in the general surgery department between October 2011 and October 2013. The diagnosis was histologically confirmed and the tumor types were classified according to 2013 WHO guidelines. There was no age or sex limits for the selection of healthy volunteers, who had no chronic diseases, lived in the same geographic area, and had no history of any cancer. All cases and controls were born and residing in Turkey.

DNA isolation

Two milliliter peripheral blood samples were obtained and collected in citrate containing tubes from all subjects. As soon as the samples reached the laboratory, DNA was extracted from whole blood by the salting-out procedure [21].

Genotyping of MDM2 SNP309 polymorphism

The distribution of polymorphisms was examined using the PCR-RFLP method. To amplify the *MDM2* SNP309 polymorphism, we used forward primer 5'-CGCGGG AGT TCA GGG TAA AG-3' and reverse primer 5'-CTG AGT CAA CCT GCC CACTG-3'. Amplification was done with 25 pmol of each primer, 200 mM total dNTP, 1.5 mM $MgCI^2$, 1xPCR buffer, 2.5 U Taq DNA polymerase, and 50-100 ng DNA in a total volume of 50 µL. The PCR program was initiated with denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 60 s, 55°C for 60 s (annealing), and 72°C for 60 s (extension). The PCR was completed with a final extension cycle at 72°C for 5 min. PCR amplification was confirmed with 1.5% agarose gel electrophoresis and then the amplified products were digested overnight with *MspIA1* restriction enzyme at 37°C, electrophoresed on 3% agarose gel, stained with ethidium bromide, and visualized under UV illumination. Genotypes for the polymorphism were identified as *TT* (157 bp), *TG* (157, 110, 47 bp), or *GG* (110, 47 bp) [18].

Statistics

All statistical analyses were performed using the Statistical Package for Social Sciences Program (SPSS, version 11). Genotype-related odds ratios (OR), 95% confidence intervals (CI), and p values were estimated via unconditional logistic regression. $P<0.05$ suggested statistical significance. Differences in the distributions of demographic characteristics between the cases and controls were evaluated using the Student's t-test. Fischer's exact test (two-sided) or x^2 were used to compare gender distribution, to test the association between the genotypes and alleles in relation to the controls, and to test

Table 1. Demographics of controls and colorectal cancer cases recruited in the study

Demographics	*Controls n (%)*	*CRC n (%)*
Sample size	145	135
Sex		
Males	86(59.3)	75(55.6)
Females	59(40.7)	60(44.4)
Age (year)		
Range	50-90	34-85
Mean±SD	63.12±8.05	63.59±11.21
Males	62.30±8.62	63.09±11.14
Females	64.32±7.03	64.22±11.35
Smoking history		
Smoker	50(34.5)	44(32.6)
Males	47(54.7)	41(54.7)
Females	3(5.1)	3(5.0)
Alcoholic drink consumption		
Yes	9(6.2)	18(13.3)
Males	9(10.5)	15(20.0)
Females	0	3(5.0)
Family history of cancer	36(24.8)	17(12.6)

for deviation of the genotype distribution from Hardy-Weinberg equilibrium (HWE). Pearson's x^2 test was used to determine whether there was any significant difference in allele and genotype frequencies between cancer cases and controls. To assess the interaction between age, gender, and genotypes (*TT*, *TG*, and *GG*), logistic regression was performed. We estimated the recessive or dominant effect of *MDM2* genotype on the relative risk of the *GG* genotype against the *TG+TT* genotype and the *TG+GG* genotype against the *TT* genotype.

Results

Polymorphism in *MDM2* SNP309 was identified in 135 cases with CRC and 145 healthy controls. The demographic characteristics of the study population are presented in Table 1. The mean age of the CRC cases and controls was 63.59±11.21 years (males: 63.09±11.14; females: 64.22±11.35) and 63.12±8.05 years (males: 62.30±8.62; females: 64.32±7.03), respectively. There were no statistically significant associations with age in either group (p=0.686). The percentages of the CRC cases of males and females in CRC cases were 55.6% and 44.4%, respectively (Table 1). There were no significant differences between cases and controls for sex (p=0.525). There was no significant association between cancer and controls for smoking (OR=0.91, CI=0.56–1.51, x^2=0.11, p=0.738; Table 2). There was a statistically significant difference for those with alcohol consumption (OR=2.32, CI=1.1–5.37, x^2=4.07, p=0.044). After adjusting for age, sex and smoking, logistic regression analysis revealed that alcohol consumption increased CRC risk (OR=2.8 95%, CI=1.1–6.7, p=0.025). There was a statistically significant difference for those with family histories of cancer (OR=0.43, CI=0.23–0.82, x^2=6.82, p=0.009; Table 2). The genotype frequencies of *MDM2* SNP309 in cancer patients were 11.9% *TT*, 68.1% TG, and 20.0% *GG*, which were statistically different from the control group: 20.0% *TT*, 57.9% TG, and 22.1% *GG* (Table 3). Finally, we found a statistically significant association between CRC risk and the *TG* genotype (OR=1.98, CI=1.1–3.91, x^2=4.00, p=0.045; Table 3). The analysis of *MDM2* SNP309 showed the following genotypes: *TT* (157 bp), *TG* (157, 110, 47 bp) and *GG* (110, 47 bp).

Table 2. Distribution of selected variables in colorectal cases and controls

Variables	*CRC (n =135)*		*Controls (n= 145)*		*p value*	*OR*
	n	*%*	*n*	*%*		
Smoking status						
No	91	67.4	95	65.5	0.738	0.91(0.56-1.51)
Yes	44	32.6	50	34.5		
Drinking status						
No	117	86.7	136	93.8	0.044	2.32(1.1-5.37)
Yes	18	13.3	9	6.2		
Family history of cancer						
No	99	75.2	128	87.4	0.009	0.43(0.23-0.82)
Yes	36	24.8	17	12.6		

Table 3. Stratification analyses between MDM2 SNP309 genotypes and colorectal cancer risk

Genotypes	*Controls n=145(%)*	*CRC n=135(%)*	x^2	*p*	*Crude OR*
Allele frequency					
T	142	124	-	-	Reference
G	148	146	0.52	0.471	1,13(0.80-1.60)
Genotype frequency					
TT	29(20.0)	16(11.9)	-	-	Reference
TG	84(57.9)	92(68.1)	4.00	0.045	1.98(1.1-3.91)
GG	32(22.1)	27(20.0)	1.09	0.295	1.52(0.68-3.39)
TG +GG	116(80.0)	119(88.1)	3.44	0.064	1.85(0.95-3.60)
TT+TG	113(77.9)	108(80.0)	0.18	0.671	1.13(0.63-2.01)

Discussion

CRC is one of the most common neoplasms in the world and is becoming more common, especially in industrialized countries. Both genetic and environmental factors are known to contribute to the increase in CRC occurrence [22-24]. Classical epidemiology has identified populations at high risk such as users of tobacco products [25]. Some authors have reported that *MDM2* SNP309 was associated with CRC risk in smokers. Terry et al. [26] found that *MDM2* SNP309 had a direct connection with CRC risk in smokers but not in non-smokers. Long-term smoking has been reported as a risk factor for CRC. However, in our study, there was no statistically significant difference in CRC occurrence between smokers and non-smokers (OR=0.91, CI=0.56–1.51, x^2=0.11, p=0.738). In a pooled analysis of eight studies in North America and Europe, a consumption of ≥45 g of alcohol per day was associated with a 1.4-fold increase in the risk of CRC [27]. A positive association between alcohol and CRC has also been observed in Asian countries [28] with few exceptions. However, the biological mechanisms between alcohol consumption and CRC remain unclear [29]. Our study also found a statistically significant association between alcohol use and CRC risk (OR=2.32, CI=1.1–5.37, x^2=4.07, p=0.044).

Genetic factors play a key role in CRC predisposition as well as the initiation and progression of the disease. High-penetrance mutations in several genes confer predispositions to familial cases of CRC, which account for less than 5% of all CRC cases [30]. Therefore, genetic variants that interfere with the *p53* cellular stress response pathway might function as modifiers of individual CRC risk. The intracellular level of *p53* is regulated through an autoregulatory feedback loop: *p53* induces the transcription of MDM2, which encodes a ubiquitin protein ligase that regulates the stability of *p53* by targeting it for proteasomal degradation [15]. On the other hand, *p53* can transactivate the *MDM2* promoter and elevate the expression of *MDM2* [31]. In response to cellular stress, such as DNA damage, *p53* expression is up-regulated; however, overexpression of *MDM2* may inhibit *p53* function, which enables damaged cells to escape the cell cycle checkpoint control and become carcinogenic [32]. In addition to functional evidence, numerous studies have demonstrated the joint effect of *p53* and *MDM2* in the carcinogenesis of human cancers [7-11]. Bond and colleagues reported a functional polymorphism in the *MDM2* promoter region, referred to as SNP309 (a T to G change at the 309th nucleotide in the first intron, rs2279744), showing a greater affinity of the transcription factor Sp1 and a higher expression of mRNA and protein of *MDM2* in the *GG* genotype than the *TT* genotype [14]. Over the last two decades, a number of molecular epidemiological studies have investigated the association between the *MDM2* SNP309 polymorphism and CRC risk, but the results remain inconsistent. Cao et al. [33] showed that the *MDM2* SNP309 polymorphism might be a risk factor for CRC, as the variant genotype was associated with significant increased CRC risk in overall populations (for *TG* and *TT*, OR=1.19, 95% CI=1.06-1.35) and in Asians (for *TG* and *TT*, OR=1.28, 95% CI=1.10-1.50). Liu et al. [34] found that increased CRC risk was more associated with the *MDM2* genotypes *GG* (OR=2.06, 95% CI=1.62-2.62) and *TG* (OR=1.31, 95% CI=1.06-1.62) than *TT*. In our study, we found a significant association between the *TG* genotype and colorectal cancer cases (OR=1.98, 95% CI=1.98-3.91, x^2=4.00, p=0.045). However, we did not find any relationship between the *GG* genotype and CRC risk (Table 3). Fang et al.'s study [35], performed almost at the same time and with similar methods, drew the opposite conclusion. The authors argue that the *MDM2* SNP309 polymorphism played a protective role in CRC susceptibility in Asians (for *GG* and *TT*, OR=0.51, 95% CI=0.41-0.64; for *GG* and TG, OR=0.64, 95% CI=0.53-0.78; for *GG*+*TG* and *TT*, OR=0.59, 95% CI=0.49-0.71; for *GG* and *TG*+*TT*, OR=0.69, 95% CI=0.57-0.82). Similarly, Wang et al. [36] found that *MDM2* SNP309 (TG/*GG*) Asians carriers had higher CRC risks (OR=1.20, 95% CI=1.03-1.38). There were significantly increased CRC risks in Asians with *TG* (OR=1.20, 95% CI=1.03-1.40) or *GG* (OR=1.21, 95% CI=1.01-1.45), when compared to *TT*. These studies failed to find a measurable association between the SNP309 G allele and CRC risk, but one study did suggest that greater CRC risk was associated with the SNP309 G allele [37]. We did not find an association between *MDM2* SNP309 and CRC risk for the *TG* genotype when individuals with the TG+*GG* genotype were compared to individuals with *TT* genotype (OR=1.85, 95% CI=0.95-3.60, p=0.06). Likewise, we did not find an association between *MDM2* SNP309 and CRC risk for the *GG* genotype when compared to the TG+*TT* genotype (OR=1.13, 95% CI=0.78-2.68, p=0.671; Table 3). Data presented in our study show that the Turkish population has a similar *GG* genotype frequency as seen in China [30], but a considerably lower frequency was found in the USA [22,38]. We observed a lower *TG* genotype frequency than that found in Finland, where populations have higher frequencies than those in Turkey [39,40].

Finally, *MDM2* SNP309 polymorphism in CRC cases has been investigated in several different regions of the world, in addition to one previous

study in Turkey. Our sample size was much larger, which is very important for making more precise estimates in epidemiological studies. In conclusion, our results show a significant relationship between the *MDM2 TG* genotype and CRC. However, it is impossible to conclude that a single polymorphism determines an individual's susceptibility to CRC. In future studies, the *MDM2 TG* genotype could be treated as an independent marker for CRC.

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Conflict of interests

The authors declare no conflict of interests.

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